REMARKS

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested.

The March 22, 2004, personal interview between Examiner Li and applicants' undersigned attorney is gratefully acknowledged. The substance of that interview is summarized below.

The rejection of claims 2-25 under 35 U.S.C. § 112 (1^{st} para.) for lack of enablement is respectfully traversed.

It is the position of the U.S. Patent and Trademark Office ("PTO") that the claimed invention is enabled only for a mouse model of pemphigus vulgaris using immune cells from a donor deficient in autoantigen Dsg3. The PTO has based this position on the assertion that "making the donor knockout animal is still unpredictable" (*see* Advisory Action mailed March 24, 2004, lines 1-2).

Applicants respectfully submit that the claimed invention is fully enabled by the present application. Moreover, as demonstrated below, based on the accompanying Second Declaration of Masayuki Amagai Under 37 C.F.R. § 1.132 ("Amagai Declaration"), as well as the exhibits referenced therein, applicants submit that one skilled in the art could readily make and use the present invention from the disclosure of the present application, without undue experimentation.

For example, the autoantigen knockout mouse line lacking desmoglein 3 ("Dsg3"), the target antigen for pemphigus vulgaris disclosed in the working examples in the present application, was prepared by a method well-known in the art (Amagai Declaration ¶ 6). Briefly, this involved creating a target vector to delete the coding sequence from the first exon of the *Dsg3* gene by homologous recombination, thereby inactivating the gene (*Id.*). To carry this out, a vector containing a neo-cassette was inserted in place of a portion of the 3' end of the first exon of a *DSG3* molecule in a mouse embryonic stem ("ES") cell (*Id.*). An ES clone with a targeted allele was injected into a mouse blastocyst (*Id.*) Chimeric F1 mice were produced from the ES clone (*Id.*). F1 mice detected by Southern blot to be positive for the targeted mutation (*i.e.*, Dsg3+/-) were intercrossed to produce F2 mice homozygous for the mutation (*i.e.*, *Dsg3-/-*) (*Id.*). mRNA expression tests for Dsg3 and immunofluorescence western blots using antibodies to Dsg3 were carried out to demonstrate that the *Dsg*3-/- homozygous mice did not produce any detectable Dsg3 protein (*Id.*). Because the *Dsg3-/-*

homozygous mice produce no Dsg3 protein, they are highly suitable for use in the present invention (*Id.*).

The working example disclosed in the present application is sufficiently illustrative such that other autoimmune/autoantigen combinations would be readily apparent to one skilled in the art (Amagai Declaration ¶ 7). Furthermore, this approach is widely applicable to various antibody-mediated and T cell-mediated autoimmune diseases (*Id.*). For example, many autoimmune diseases were well characterized at the time the invention was made (*Id.*). Indeed, at the time the invention was made, numerous autoantigens and their genes had been positively identified (*Id.*). Examples of specific autoimmune disease/autoantigen combinations known in the art at the time the invention was made are shown in Table 1 (*Id.*). In addition, a knockout animal deficient in each of the genes described in Table 1 has been made and shown to be viable (*Id.*). This list, while not exhaustive, is indicative of the advanced state of the art in autoimmune disease identification, as well as that of applicable knockout technology, at the time the present invention was made (*Id.*).

Furthermore, the ability to create knockout animals has been known and practiced for many years (Amagai Declaration ¶ 8). In addition to several of the references cited above, a multitude of publications were available at the time the present invention was made that describe, in detail, successful procedures for producing knockout animals (*Id.*). Rajewsky et al., "Molecular Medicine in Genetically Engineered Animals," *J Clin Invest* 98(3):600-603 (1996), for example, teaches a method of producing knockout animals by mutagenesis that involves the use of the bacteriophage *Cre*-loxP recombination system to produce gene deletions, replacements, insertions, and even cell- and tissue-specific mutations that has been shown to work well in mice (*Id.*). Given the detailed guidance for making knockout animals that was available when the present invention was made, combined with the high degree of success described therein, it is apparent that a skilled scientist would have known how to make other autoantigen donor animals with which to practice the present invention.

The production of knockout animals, mice and rats in particular, is a technique that is highly valuable for studying genetic function and the process of disease (Amagai Declaration ¶ 9). Furthermore, given the refinement of genetic engineering techniques over the last 20 years, it is expected that knockout mice will continue to be an invaluable experimental tool in basic and applied research (*Id.*).

Turning to the question of whether the effect of the knockout on the viability of the resulting phenotype creates a barrier to practicing the present invention, it is clear that the present invention is not applicable to the study of all autoimmune diseases and/or all autoantigens (Amagai Declaration ¶ 10). Indeed, some autoantigen knockout animals may not be viable (*Id.*). However, one skilled in the art would readily recognize that the "antigen of an autoimmune disease" cannot constitute an essential protein if the knockout animal is to be viable (*Id.*). Furthermore, two generalizations have emerged from examining knockout mice: 1) knockout mice are often surprisingly unaffected by their deficiency, as many genes turn out not to be indispensable; 2) most genes are pleotropic, *i.e.*, they are expressed in different tissues in different ways and at different times in development (*Id.*). Thus, one skilled in the art would have a high expectation of producing a viable knockout animal using the techniques known in the art. Moreover, a skilled scientist can distinguish between operative and inoperative embodiments with only minimal effort. A skilled scientist would also know that the problem of lethal homozygous knockouts can be addressed using a modification of the *Cre*-lox recombination method, a technique that involves introducing a construct into the null background to rescue the developmental defect, but not the downstream functions of the deleted gene (*Id.*). In addition, because the *Cre*-lox system removes the need for the selection marker DNA sequence(s) required by the classical homologous recombination method of knockout production, the potential for unexpected mutant phenotypes can be reduced or removed by using the *Cre*-lox knockout production technique as an alternative to homologous recombination (*Id.*).

The technique of "adoptive transfer," in which immune cells from a donor of interest are transplanted to a host in which the immune responsiveness has been eliminated, is a well-known method used for the study of immune cells and of the cellular interactions required in an immune response (Amagai Declaration ¶ 11). The literature is replete with reports in which adoptive transfer has been used to transfer immune cells to a host individual for immunological investigations of disease conditions and for *in vivo* therapeutic treatment (*Id.*). Therefore, one of ordinary skill in the art would have been fully familiar with adoptive transfer techniques and would have a high expectation of success when used in accordance with the disclosure of the present patent application (*Id.*).

Moreover, the claimed recipient animal need not exhibit a phenotype corresponding to a particular autoimmune disease (Amagai Declaration ¶ 12). Rather, all that is required by the claimed invention is that the recipient animal "produces an antibody

reactive to the antigen protein and/or has activated T cells reactive to the antigen protein" (*see* claims 2 and 3) (*Id.*). Therefore, the display of symptoms or characteristics of a particular autoimmune disease is not an element of the claimed invention (*Id.*). Rather, an essential feature of the present invention is the creation of an animal that produces an antibody against a self-antigen and the use of such an animal in the study of autoimmune conditions (*Id.*). Even in autoimmune diseases that involve multiple antigens, the instant invention remains applicable for producing antibodies against one or a few autoantigens, thereby inducing an autoimmune response against these antigens in the recipient (*Id.*). Therefore, in this respect, autoimmune animal models made according to the present invention would have great utility in the investigation of disease states and potential treatments thereof (*Id.*).

In assessing the degree of experimentation required to practice the present invention, it should be considered, first of all, that the preparation of knockout animals has reached a level of high predictability as evidenced by the availability of specific vectors designed for the preparation of knockouts, the rise of commercial enterprises that offer preparation of knockout animals on an as-needed basis, and the numerous peer review journals that describe making and/or using knockout animals, including, but limited to, those named in the Amagai Declaration (Amagai Declaration ¶ 13). Secondly, it is important to note that a major hurdle in developing animal models of autoimmune diseases has been overcoming the self-tolerance component of the homeostatic system (*Id.*). An example of this problem is provided in the present application at Example 2, which describes applicants' initial failure in producing an autoimmune response using the conventional multiple injection technique in a wild-type (*i.e.*, *Dsg3*+/+) mouse (*Id.*). The present invention circumvents this long-standing problem by immunizing autoantigen knockout mice with the autoantigen, then transferring their splenocytes to mice that express the autoantigen (*Id.*). Thus, the effort in making an autoantigen knockout animal is offset by the tremendous advantage to be gained over prior art methods in using the present invention for making an autoimmune model without the complications of antigen self-tolerance (*Id.*).

Therefore, given the knowledge of a skilled scientist at the time the present invention was made as to the identification and characterization of numerous autoimmune diseases and the responsible autoimmune/autoantigen combinations, the availability of successful, predictable methods for production of knockout animals, rodents in particular, a skilled scientist, having read the present application, would be fully able to prepare or obtain,

without undue experimentation, an autoimmune antigen knockout animal suitable for use in the present invention (Amagai Declaration ¶ 14).

For all of the above reasons, the rejection of claims 2-25 under 35 U.S.C. § 112 (1st para.) for lack of enablement is improper and should be withdrawn.

In view of all the foregoing, applicants submit that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

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